

Claims

1. An isolated nucleic acid molecule coding the expression box with the formula:
 S_1 - S_2 - S_3

wherein:

S_1 is a promoter sequence, or it is absent,

S_2 is a known reporter gene sequence,

S_3 is a regulatory 3'UTR sequence, or it is absent,

where the promoter sequence and the regulatory 3'UTR sequence originate from a known cytokine gene, and are the controlling sequences of said cytokine.

2. A nucleic acid molecule according to claim 1, characterised in that the reporter gene is a gene coding a Green Fluorescent Protein (GFP), possibly selected from its variants: d1EGFP, d2EGFP, EGFP or EGFP-F.

3. A nucleic acid molecule according to claim 1, characterised in that the promoter sequence and regulatory 3'UTR sequence originate from a cytokine selected from among the following: IL-1 β , IL-2, TNF α , IL-4, IL10 or INF γ .

4. A nucleic acid molecule according to claim 1, characterised in that it is an expression box contained in a plasmid selected from among the following: p1-5'IL1 β /d1EGFP-N1 (SEQ ID NO:1), p2-5'IL1 β /d1EGFP-N1 (SEQ ID NO:2), p3-5' IL1 β /d1EGFP-N1 (SEQ ID NO:3), p4-5'IL1 β /d1EGFP-N1 (SEQ ID NO:4), p1-5'3' IL1 β /d1EGFP-N1 (SEQ ID NO:5), p2-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:6), p3-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:7), p4-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:8), p1-5'IL2/EGFP-1 (SEQ ID NO:9), p1-5'IL2/d2EGFP-1 (SEQ ID NO:10), p1-5'3'IL2/d2EGFP-1 (SEQ ID NO:11), p1-3'TNF α /d1EGFP-N1 (SEQ ID NO:12), p2-3'TNF α /EGFP-F (SEQ ID NO:13), p3-3'TNF α /EGFP-F (SEQ ID NO:14), p1-5'TNF α /d1EGFP-N1 (SEQ ID NO:15), p1-5'3'TNF α /d1EGFP-N1 (SEQ ID NO:16), p1-3'IL4/d1EGFP-N1 (SEQ ID NO:17), p2-3'IL4/EGFP-F (SEQ ID NO:18), p3-3'IL4/EGFP-F (SEQ ID NO:19), p4-3'IL4/CA-EGFP (SEQ ID NO:20), p5-3'IL4/d1EGFP-N1 (SEQ ID NO:21), p1-5'IL4/EGFP-1 (SEQ ID NO:22), p1-5'IL4/d1EGFP-N1 (SEQ ID NO:23), p2-5'IL4/EGFP-1 (SEQ ID NO:24), p2-5'IL4/d1EGFP-N1 (SEQ ID NO:25), p1-5'3'IL4/EGFP-1 (SEQ ID NO:26), p1-5'3'IL4/d1EGFP-N1 (SEQ ID NO:27), p2-5'3'IL4/d1EGFP-N1 (SEQ ID NO:28), p1-5'INF γ /EGFP-1 (SEQ ID NO:29), p1-5'INF γ /d2EGFP-1 (SEQ ID NO:30), p1-5'3'INF γ /d2EGFP-1 (SEQ ID NO:31), p1-5'IL10/EGFP-1 (SEQ ID NO:32), p1-

5'3'IL10/EGFP-1 (SEQ ID NO:33), p2-5'IL10/d2EGFP-1 (SEQ ID NO:34), p2-5'3'IL10/d2EGFP-1 (SEQ ID NO:35).

5. An expression vector containing a nucleic acid molecule coding an expression box with the formula:

S1-S2-S3, wherein

S1 is a promoter sequence, or it is absent,

S2 is a known reporter gene sequence,

S3 is a regulatory 3'UTR sequence, or it is absent,

where the promoter sequence and the regulatory 3'UTR sequence originate from a known cytokine gene, and are the controlling sequences of said cytokine.

6. An expression vector according to claim 5, characterised in that the reporter gene is a gene coding a Green Fluorescent Protein, possibly selected from its variants: d1EGFP, d2EGFP, EGFP or EGFP-F.

7. An expression vector according to claim 5, characterised in that the promoter sequence and regulatory 3'UTR sequence originate from a cytokine selected from among the following: IL-1 β , IL-2, TNF α , IL-4, IL10 or INF γ .

8. An expression vector according to claim 5, characterised in that it is a plasmid selected from among the following: p1-5'IL1 β /d1EGFP-N1 (SEQ ID NO:1), p2-5'IL1 β /d1EGFP-N1 (SEQ ID NO:2), p3-5' IL1 β /d1EGFP-N1 (SEQ ID NO:3), p4-5'IL1 β /d1EGFP-N1 (SEQ ID NO:4), p1-5'3' IL1 β /d1EGFP-N1 (SEQ ID NO:5), p2-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:6), p3-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:7), p4-5'3'IL1 β /d1EGFP-N1 (SEQ ID NO:8), p1-5'IL2/EGFP-1 (SEQ ID NO:9), p1-5'IL2/d2EGFP-1 (SEQ ID NO:10), p1-5'3'IL2/d2EGFP-1 (SEQ ID NO:11), p1-3'TNF α /d1EGFP-N1 (SEQ ID NO:12), p2-3'TNF α /EGFP-F (SEQ ID NO:13), p3-3'TNF α /EGFP-F (SEQ ID NO:14), p1-5'TNF α /d1EGFP-N1 (SEQ ID NO:15), p1-5'3'TNF α /d1EGFP-N1 (SEQ ID NO:16), p1-3'IL4/d1EGFP-N1 (SEQ ID NO:17), p2-3'IL4/EGFP-F (SEQ ID NO:18), p3-3'IL4/EGFP-F (SEQ ID NO:19), p4-3'IL4/CA-EGFP (SEQ ID NO:20), p5-3'IL4/d1EGFP-N1 (SEQ ID NO:21), p1-5'IL4/EGFP-1 (SEQ ID NO:22), p1-5'IL4/d1EGFP-N1 (SEQ ID NO:23), p2-5'IL4/EGFP-1 (SEQ ID NO:24), p2-5'IL4/d1EGFP-N1 (SEQ ID NO:25), p1-5'3'IL4/EGFP-1 (SEQ ID NO:26), p1-5'3'IL4/d1EGFP-N1 (SEQ ID NO:27), p2-5'3'IL4/d1EGFP-N1 (SEQ ID NO:28), p1-

5'INF γ /EGFP-1 (SEQ ID NO:29), p1-5'INF γ /d2EGFP-1 (SEQ ID NO:30), p1-5'3'INF γ /d2EGFP-1 (SEQ ID NO:31), p1-5'IL10/EGFP-1 (SEQ ID NO:32), p1-5'3'IL10/EGFP-1 (SEQ ID NO:33), p2-5'IL10/d2EGFP-1 (SEQ ID NO:34), p2-5'3'IL10/d2EGFP-1 (SEQ ID NO:35).

9. A single-celled host transformed or transfected with a DNA molecule according to one of claims 1 to 4.
10. A single-celled host according to claim 9, characterised in that it is transformed or transfected with a vector according to one of claims 5 to 8.
11. A single-celled host according to claim 9, characterised in that it is selected from the group encompassing bacteria, yeast, mammalian cells, plant cells, insect cells, as well as eukaryotic cell lines.
12. A single-celled host according to claim 11, characterised in that it is an immortal mammalian cell line, preferentially descendant from cells of the immune system.
13. A single-celled host according to claim 11, characterised in that it is a cell line selected from among T cell leukemia cells, thymoma, mast cells, macrophage-monocytes, fibroblasts and keratinocytes.
14. A single-celled host according to claim 12, characterised in that as a result of recombination, the natural cytokine gene extant in the host cell has been replaced by the DNA molecule according to one of claims 1 to 4.
15. A single-celled host according to claim 11, characterised in that it is a cell line selected from among the following: EL4, BW5147.3, C57.1, J774A.1, 3T3 L1, MC/9 and HEL-30.
16. A single-celled host according to claim 11, characterised in that it is a cell line selected from among C/p1-5'3'TNF α -dEGFP/2 (deposited in ECACC, Accession No. 3091202), EL/p1-5'IL2-dEGFP/6 (deposited in ECACC, Accession No. 3091204), EL/p2-5'IL4-dEGFP/2 (deposited in ECACC, Accession No. 3091205), EL/p1-5'IFN γ -dEGFP/3 (deposited in ECACC, Accession No. 3091206), EL/p2-5'IL10-dEGFP/5 (deposited in ECACC, Accession No. 3091207), J/p4-5'IL1 β -dEGFP/4 (deposited in ECACC, Accession No. 3091208).

17. A collection of cell lines characterised in that it contains at least one cell line according to one of claims 9 to 16 as well as at least one positive control cell line showing a constitutive expression of the reporter gene sequence.
18. A collection of cell lines according to claim 17, characterised in that the positive control cell line originates from cells selected from a group encompassing bacteria, yeast, mammalian cells, plant cells, insect cells, as well as eukaryotic cell lines.
19. A collection of cell lines according to claim 18, characterised in that the positive control cell line is an immortal mammalian cell line.
20. A collection of cell lines according to claim 17, characterised in that in the positive control cell line the reporter gene sequence is operationally bound to the regulatory sequence giving constitutive expression, where possibly it contains at least one element from among the following: 3'UTR GAPDH, promoter/enhancer CMV, promoter-actin or their derivatives.
21. A collection of cell lines according to claim 17, characterised in that the positive control cell line is transformed or transfected with a plasmid selected from among the following: p1-3'GAPHD/d1EGFP-N1 (SEQ ID NO:36), p2-3'GAPHD/EGFP-F (SEQ ID NO:37), p3-3'GAPDH/EGFP-F (SEQ ID NO:38), pCA-EGFP-F (SEQ ID NO:39), pCA-d1EGFP (SEQ ID NO:40).
22. A collection of cell lines according to claim 17, characterised in that the positive control cell line is the C/pCA-EGFP-F/2 line (deposited in ECACC, Accession No. 3091201) or EL/pCA-dEGFP/9 (deposited in ECACC, Accession No. 3091203).
23. A collection of cell lines according to claim 17, characterised in that it is a cell-chip.
24. A method of obtaining characteristics of the tested substance, characterised in that
- a) the tested substance is put into contact with the cell line according to one of claims 9 to 16, or a cell line belonging to a collection of cell lines according to one of claims 17 to 23,
 - b) it determines a change in the level of expression of a reporter gene caused by the tested substance,
 - c) a change in the level of expression described in (b) is accepted as characteristic of the tested substance.

25. A method according to claim 24, characterised in that GFP or one of its known variants is used as a reporter gene, and in stage (b) changes in the intensity of fluorescence are measured.
26. A method according to claim 24, characterised in that in (b) changes in the level of expression of the reporter gene is studied for each cell line in the collection.
27. A method according to claim 24, characterised in that in (c) an expression profile characterizing the tested substance is obtained, based on results obtained from cell lines belonging to the collection.
28. A method according to one of claims 24 to 27, characterised in that stage (a) is performed on all cell lines belonging to the collection.
29. A method according to one of claims 24 to 27, characterised in that stage (b) is performed on all cell lines belonging to the collection.
30. A method according to one of claims 24 to 27, characterised in that stages (a) and (b) are performed automatically.
31. A method according to one of claims 24 to 30, characterised in that in stage (c), the results of measurements of changes in the level of expression obtained are computer analysed.
32. A method according to one of claims 24 to 31, characterised in that in stage (c) the results obtained from the tested substance are compared to results obtained from substances of known properties.
33. A method according to one of claims 24 to 32, characterised in that stage (a) is performed in the presence of an expression modulator.
34. A method according to claim 33, characterised in that the modulator of expression is an activator inducing the expression of the reporter gene.
35. A method according to claim 34, characterised in that the activator is selected from among the following: PMA, ionomycin, calcium ionophore, LPS or their combinations.
36. A method according to one of claims 24 to 35, characterised in that the characteristics obtained in stage (c) are used to ascertain the biological activity of the tested substance.
37. A method according to claim 36, characterised in that in stage (c) the results of expression level measurements obtained from the tested substance are compared to results obtained from a reference substance of known biological activity.

- 38. A method according to claim 36, characterised in that the studied biological activity is toxicity.
- 39. A method according to claim 36, characterised in that the studied biological activity is immunotoxicity.
- 40. Use of a cell line according to one of claims 9 to 16 or a collection of cell lines according to claims 17-23 to study the biological activity of the tested substance.
- 41. Use according to claim 40, characterised in that the studied biological activity is toxicity.
- 42. Use according to claim 40 or 41, characterised in that the studied biological activity is immunotoxicity.
- 43. Use of a cell line according to one of claims 9 to 16 or a collection of cell lines according to claims 17-23 to obtain the characteristics of the tested substance.